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Mesenchymal Stem Cells and Senescence

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Abstract

Mesenchymal stem cells (MSCs) have the ability of self-renewal and differentiation into multiple tissues. The senescence of MSCs may involve some age related disease and autoimmune disease such as SLE. Senescent MSCs may limit MSCs clinical application. Thus, analysis of *in vitro* senescence in MSC is crucial for quality control of MSCs preparations used for therapeutic application. This review summarizes recent advances about main characteristics of senescent MSCs, molecular mechanisms and signal pathway of MSCs senescence.

Mesenchymal stem cells (MSCs) have the ability of self-renewal and differentiation into multiple tissues. Unlike other stem cells, MSCs have characteristics of a low expression of major histocompatibility complex class I and the absence of co-stimulatory molecules such as CD80, CD86 or CD40. Therefore, there is no problem with tissue matching and immune rejection when transplantation of MSCs in treatment of certain autoimmune diseases. MSCs have some significant effects on immune modulation. MSCs modulate both innate and adaptive immunity, and such properties are promising use to clinical applications directly. Due to their unique biological and immunological properties, MSCs raise high hopes for cell-based clinical therapies and their use is concurrently tested in various clinical trials . MSCs-based therapies may be an interesting way for autoimmune diseases and inflammatory, as supported by approximately 3500 trials on adult stem cells. Recently, several research have demonstrated the efficacy of expanded MSCs infusion for diseases including Acute Graft Versus Host Disease (*GVHD*), osteoarthritis, Crohn's disease, Liver diseases, Diabetes, Heart diseases, Limb ischemia, SLE and Neurological diseases .

However, several factors influence the effective use of MSCs in clinical application, in particular by the relatively small yield of cells from adult tissues. Some studied showed that only ~2000 cells were isolated from one bone marrow aspirate at times and the number of MSCs is quite low for and infusion and cell-based clinical therapies. Therefore, the development of techniques to obtain a sufficient number of primary cells and the study for effective expansion were a prerequisite for MSCs therapeutic approaches.

Keywords: Mesenchymal stem cells; Senescence; Self-renewal; Differentiation

Characteristics of Mesenchymal Stem Cells

Multipotent MSCs are adult mesenchymal, non-hematopoietic stem cell that can be obtained from a variety of tissues, including bone marrow, adipose-tissue, dental pulp, umbilical cord blood, and placenta. An important characteristic of MSCs is their plasticity [1-8]. MSCs have the potential to differentiate into cell derivatives of the mesenchymal lineage, including adipocytes, osteocytes, chondrocytes and myocytes [9,10]. MSCs are also characterized by potential of long term self-renewal. MSCs have been defined as a heterogeneous stem cell population by the International Society of Cellular Therapy. Three minimal criteria for the identification of MSCs: 1) MSCs must adhere to plasticity under standard tissue culture conditions; 2) expression of CD105, CD73 and CD90, and non-expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II as measured by flow cytometry; 3) MSCs must also be able to differentiate to osteoblasts, adipoblasts and chondroblasts *in vitro* [11].

Additional, MSCs exhibit immunosuppressive and healing capacities by improving angiogenesis and preventing apoptosis and/or fibrosis through the secretion of paracrine mediators [12]. MSCs were able to home the sites of inflammation following tissue injury after injected intravenously. MSCs secreted a large spectrum of bioactive molecules and these factors will promote tissue regeneration and the damaged tissue repair. The production factors by MSCs provide benefit through: increased proliferation and surivival of endogenous cells; induced angiogenesis; anti-inflammatory effects; anti-apoptotic effects; immunomodulatory effects and even the transfer of mitochondria [13-15].

Main Characteristics of Senescent MSCs

MSCs have a limited number of divisions *in vitro* as any normal somatic cells before entering a state of replicative senescence. In this state, they are morphologically characterized by flattened and enlarged cell shapes. They stop dividing further but remain viable and increase expression of senescence-associated β-galactosidase (SA-β-Gal). Leonard Hayflick first described this phenomenon in the 1960s [16]. The characteristics of the same origin senescent MSCs are very similar. The expression of specific surface markers of senescent MSCs attenuated when compared to early-passage cells, but their qualitative characteristics do not reveal the presence of any distinct epitopes [17]. Senescent MSCs display an excess of actin stress fibers, decreased adherence to plastic surfaces, and increased auto-fluorescence level, which is a sign of lipofuscin accumulation [18]. Previous studies showed that in prolonged MSCs cultivation of SA-β-Gal activity increases [19]. The percentage of SA-β-Gal positive cells in the last passages of MSCs cultures reaches approximately 80%, which indicates about a four-fold increase throughout cell lifespan [17,19]. Altered expression of senescence-associated gene is in line with the changes in morphology and cell biology. Also, in murine MSCs molecular genetic changes happen during aging and are then conserved during passage in culture, likely affecting the physiological functions and the potential of autologous MSCs for stem cell therapy [20]. Thus, analysis of *in vitro*

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senescence in MSC is crucial for quality control of MSCs preparations used for therapeutic application.

Molecular Mechanisms of MSCs Senescence

Various effectory mechanisms involve MSCs senescence. Shortening of telomere length, decreased telomerase activity, loss of genomic and mitochondrial genomic integrity due to DNA damage and progressive loss of DNA repair ability and epigenetic alternation have all been suggested to contribute to senescence [21]. As in lymphocytes, amniocytes and fibroblasts, the telomeres of MSCs have a specific and largely conserved telomere length pattern on individual chromosome ends [22]. One of the molecular mechanisms is believed to be co responsible for senescence of MSCs such as the lack of a detectable telomerase activity [23,24]. It has been postulated that progressive shortening of the telomeres or modified telomeric structure has been correlated with replicative senescence [25,26].

High expression of p53 and its downstream target $p21^{Cip1}$ triggered a telomere-dependent manner, growth cessation in senesce cells [27]. The action of tumour suppressor p53 in cell cycle regulation is to keep the cell from progressing through the cell cycle when DNA damage is present, either by keeping the cell at a checkpoint until repairs are made, or by causing the cell to enter apoptosis when the damage cannot be repaired [21,28]. There is also evidence that senescence involves oxidative stress DNA, strand breaks, and accumulation of the cyclin-dependent kinase inhibitor $p16^{INK4a}$ [29-31]. The $p16^{INK4a}$ involves growth arrest and induces cellular senescence, and its expression improves age-dependency, in line with the accumulation of senescent cells [32]. We have found that MSCs from SLE show senescent characteristic. We also found $p16^{INK4a}$ expression levels was increased in senescence MSCs, whereas levels of CDK4, CDK6 and p-Rb expression were decreased in senescence MSCs. Knockdown of $p16^{INK4a}$ expression reversed the senescent features of MSCs. overexpress of $p16^{INK4a}$ expression unchanged levels of p53 and $p21^{Cip1}$ in senescent MSCs [33,34]. These findings further demonstrated that $p16^{INK4a}$ expression in senescent MSCs correlates positively with the SA-β-Gal activity and negatively with proliferation marker Ki67.

One of the main reasons leading to DNA damage and cell senescence is oxidative stress [35]. In aging, an increased production of free radicals causes oxidative stress through oxidative metabolism, as such it has been indicated that lower amount of mitochondrial ROS can be detected in long-lived species [36]. The proliferation and replicative lifespan of were markedly increased after treated with antioxidants such as ascorbic acid or resveratrol [37]. Recent studies have demonstrated that ROS generation could induce cell senescence by induced DNA damage response [38].

Shortening of telomere length, decreased of telomerase activity triggered the process of MSCs senescence. The expression level of $p16^{INK4a}$ and $p53/p21^{Cip1}$ is increased in senescent MSCs (Figure 1). Cells that eventually exhaust their capacity to divide a flattened and enlarged cell shapes, increased SA-β-Gal positive cells ratio, accumulated generation of ROS, and increased content of the lipid per-oxidation product.

Signalling Involved in MSCs Senescence

In adult mammals, Wnt/β-catenin signalling is crucial for regulating cell proliferation, cell fate determination, apoptosis, and axis polarity induction [39]. Some recent studies have shown that activated Wnt/β-catenin signaling is an important mediator of MSCs aging induced by old rat serum [40]. DNA damage response and p53/p21 pathway have an important role in MSC aging induced by excessive Wnt/β-catenin signaling. DNA damage response induced cell senescence through the $p16^{INK4a}$ gene or the p53/p21 pathway [41]. Active Wnt/β-catenin signaling could improve the oxidase activity [42]. The expression of γ-H2A.X, $p16^{INK4a}$, p53, and p21 is increased in senescent MSCs induced with old rat serum, and is also reversed by the Wnt/β-catenin signalling inhibitor DKK1 or by β-catenin siRNA. The activated c-myc, a target gene of Wnt/β-catenin signalling, induces cellular senescence by promoting $p16^{INK4a}$ expression [43].

TGF-β has been found to induce cellular senescence of keratinocytes [44]. The gene expression profile analysis indicated that the expression of TGF-β1, TGF-β2, and TGF-β receptor type I increased significantly in senescent MSCs. TGF-β1, TGF-β2 inhibits MSCs growth and contributes to premature cell senescence with high expression of $p16^{Ink4a}$ and $p53/p21^{Cip1}$ [45].

FOXO transcription factors regulate downstream target genes that affect cellular differentiation, cellular survival, cellular metabolism, cell-cycle arrest and oxidative stress responses [46]. The aging process down-regulates the levels of FOXO; binding activity is enhanced through PI3K/Akt pathways. Furthermore, this age-dependent decrease of FOXO via inhibiting PI3K/Akt pathways can be blocked by calorie restriction. PI3K/Akt-mediated the activation of FOXO1 causing decreased catalase levels and increased cellular ROS. FOXO3a and FOXO4 protect quiescent cells *in vitro* from oxidative stress [47]. The phosphorylation of FOXO3 impairs the activation of FOXO3, resulting in cytoplasmic sequestration and inhibition of its downstream transcriptional activity, which produced high levels of ROS and causes senescence [48]. Recent study have shown that intracellular ROS (reactive oxygen species) levels of SLE MSCs were higher than those of normal controls, with the activation of PI3K/AKT/FOXO3 signalling pathway [48].

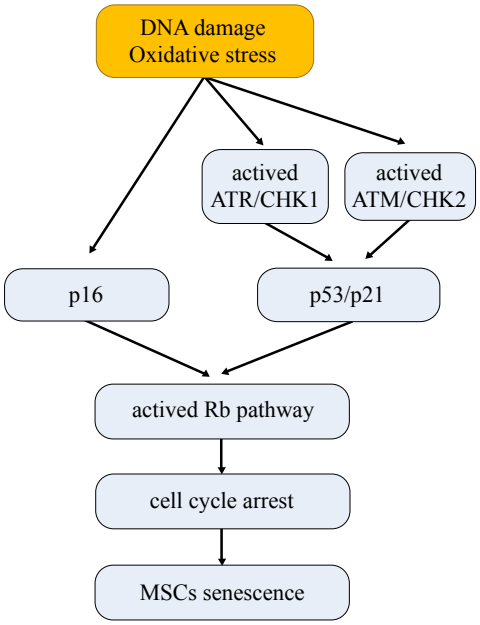

Figure 1: The possibe mechamism by which p53/p21 and $p16^{INK4a}$ modulate MSCs senescence. Oxidative stress and DNA damage induce MSCs senescence by activating the ATM/ATR pathway and Chk1/Chk2 to stabilize p53/p21. Oxidative stress can indirectly induce DNA damage. Oxidative stress and DNA damage can also cause high expression of $p16^{INK4a}$. Up-regulate the expression of p53/p21 and $p16^{INK4a}$ increase the expression of the active, hypophosphorylated Rb. Activated Rb pathway can make cells enter cell cycle phase arrest that eventually contributes to MSCs senescence.

In conclusion, the senescence of MSCs presents a big challenge for large-scale *ex vivo* expression and maintenance of MSCs stemness. The senescence of MSCs may involve some age related disease such as SLE. In this review, we summarize the characteristics of MSCs, senescent MSCs and molecular mechanisms, signalling involved in MSCs senescence. Analyzing the mechanisms of controlling MSCs senescence provides a target therapeutic application for age related disease.

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